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The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers

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Abstract

Since 2007 about 200 to 300 fish per year (generally roach (*rutilus rutilus*) but also a few bleak (*alburnus alburnus*) and eels (*anguilla anguilla*)) have been collected from a number of English river sites and stored at -80°C to build up a Fish Tissue Archive as a resource for the monitoring of pollutants. Some of the fish from the Fish Tissue Archive from the years 2007-2011 were analyzed for substances in current and proposed European legislation regarding environmental quality standards (EQS) in biota. It was found that mercury exceeded the EU EQS of 20 µg/kg in 79 % of samples with an average and median of 31 and 29 µg/kg. The legacy fungicide hexachlorobenzene (HCB) was below the EQS of 10 µg/kg in all fish analyzed, with a maximum of 6 µg/kg in some eels. The legacy solvent hexachlorobutadiene (HCBD) was below the EQS of 55 µg/kg, being < 0.2 µg/kg in all samples where it was measured. The sums of six polybrominated diphenyl ethers (PBDEs) were several orders of magnitude higher than the new proposed 0.0085 µg/kg biota EQS. This study showed that the regular collection and analysis of whole body homogenate samples of relatively small native pelagic fish is suitable for the monitoring of contaminants capable of bioaccumulation. With regard to current or proposed EQS for EU countries, mercury and potentially PBDE in fish are of some concern in these English rivers.

Keywords

Environmental quality standards

UK

fish

mercury

PBDE

hexachlorobenzene

priority substances

rivers

1 Background and introduction

1.1 Legislation

The Priority Substances Directive of the EU (Directive 2008/105/EC), which entered into force in January 2009, has the objective of protecting wildlife and humans from harmful effects of chemicals identified as priority substances in surface waters and to monitor trends of these chemicals. It aims to set environmental quality standards (EQS) for a number of chemical pollutants below which no harmful effects are expected to wildlife, or humans. There is an option for member states to set biota, or sediment, standards which offer "at least the same level of protection" as the water standards. For mercury, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD), the legislation states that water standards alone do not offer sufficient protection and therefore limits have been set for both water and biota and the legislation specifies that member states which choose not to apply the biota standard should set more stringent levels than those set out in the directive for the water standard. The EQS is set for prey tissue (wet weight) with member states being able to choose "the most appropriate indicator from among fish, molluscs, crustaceans and other biota;" (Directive 2008/105/EC, Article 3 (2a)). Where current levels exceed the EQS, downward trends should be demonstrated as "the Commission shall, by 2018, verify that emissions, discharges and losses as reflected in the inventory are making progress towards compliance […]" (Directive 2008/105/EC, Article 5(5)). At the beginning of 2012 a proposal to amend the legislation was published

(European Commission, 2012), which includes biota standards for a further eight chemicals in addition to the previous three and also states that, unless specified, the standards should be for fish rather than the more generic "prey" in the current version. Polybrominated diphenyl ethers (PBDEs) are an example of this group of chemicals for which biota standards are now proposed.

This study aimed to explore the overall practicality of bio-monitoring of wild fish to assess contamination by hydrophobic, or bioaccumulative, persistent chemicals proposed in EU Directives and to test the hypothesis that none of the chemicals with biota EQS would be exceeded in any fish caught in English rivers and that levels would not differ appreciably between sites, or species.

1.2 Chemicals of interest

Hexachlorobenzene (HCB), is a hydrophobic (reported log K_{ow} of 5.5), and highly persistent chemical (Fox et al., 2001; Verweij et al., 2004). In the UK it was used between the 1940s and 1975 as a fungicide for seed treatment. After 1975, sources were unintentional, occurring as an impurity in other chlorinated products (particularly the pesticide chlorthalonil) and forming during combustion processes involving chlorine and organic matter and in aluminum smelters, where until 2000 hexachloroethane, which can get converted to HCB, was used as a degassing agent (Conolly et al., 2010; Fox et al., 2001). In 2002 it remained a consistent and widespread atmospheric contaminant across Europe (Jaward et al., 2004). Its toxicity to humans was dramatically demonstrated in the late 1950s when thousands of people in Turkey suffered liver damage after eating HCB treated grains and many babies died as a consequence of feeding on contaminated breast milk (Gocmen et al., 1989). In terms of the toxicity to wildlife, EUROCHLOR, the trade organization of European chlorine producers, extrapolated from published water no observed effects concentrations (NOECs) to body burdens and calculated that the no observed effects level (NOEL) expressed as body burden for fish would be 7,500 µg/kg (Eurochlor, 2002), but by extrapolating from the effects of food-borne HCB on animals in laboratory studies the Niagara River Biota Project (Newell et al., 1987) derived much lower safe levels to protect fish-eating mink, estimating the NOEL for non-carcinogenic effects as 330 µg/kg in the prey fish and that a contamination of 20 µg/kg in the fish would give mink a lifetime cancer risk of 1/1000. The latter value is similar to recommendations by the US EPA which state that humans who eat food containing 29 µg/kg for 130 weeks may experience health effects (http://www.epa.gov/osw/hazard/wastemin/minimize/factshts/hexchlbe.pdf, accessed 1.5.2013) and to the EU biota standard of 10 µg/kg in "prey". The water EQS is set at 10 ng/L for annual averages and 50 ng/L as a maximum,

but as with all three substances for which biota standards currently exist, member states are required to set lower standards if they choose not to monitor biota as well.

Due to its persistency, HCB was found in some estuarine bed-sediments dating back to the very beginning of its production around 1900 (Fox et al., 2001). The Environment Agency (EA) has been monitoring HCB in water at several sites along the River Thames and one on the River Welland since 2006: Out of 136 measurements, only on one occasion was HCB above the detection limit of 1 ng/L (Environment Agency, Water Information Management System (WIMS) database, see table 1). Literature data is also well below the 10 ng/L water EQS with average concentrations in rivers of the Humber catchment (UK) 0.13-0.80 ng/L (maximum 4 ng/L) in 1996 (Meharg et al., 1998) and concentrations reported in surface waters in the Amsterdam region in 2000 about 0.02-0.1 ng/L, except for ditches near a landfill site which had concentrations in the ng/L range (Verweij et al., 2004). In fish, HCB concentrations have been monitored for example in eels in the Netherlands where they reduced about ten fold in a decade from 144-2820 µg/kg lipid weight in 1984 to 22-243 µg/kg lipid weight in 2004 (de Boer et al., 2010). In Flanders (Belgium) a reduction of HCB concentration in eels was also demonstrated for data from 1994 to 2005 and at most sites the concentrations were below the EQS throughout that period leading to an overall average of 5.9 µg/Kg wet weight (ww), but a maximum concentration of 192 µg/kg was reported (Maes et al., 2008).

Hexachlorobutadiene (HCBD), is also hydrophobic, with a reported log K_{ow} of 3.7-4.9 and considered to be a persistent chemical (Lerche et al., 2002). With a vapor pressure of 20 Pa at 20°C it is quite volatile. HCBD was mainly used as a solvent in the production of rubber and other polymers, but also in agriculture as a seed dressing, in hydraulic fluids and a number of other industrial processes (Taylor et al., 2003). Intentional production has practically ceased in the EU, but it can be formed as an unintended by-product during the production of other chlorinated substances. Improved manufacturing processes mean that today very little is released (Lecloux, 2004). Studies in rats and humans show that HCBD undergoes several metabolization steps in the body forming the highly toxic trichlorovinyl-chlorothioketene (TCCT) in the kidney where it binds to adjacent tissue, causing toxic and carcinogenic effects (Staples et al., 2003). There is little data on toxicity to wildlife but the Niagara River Biota Project (Newell et al., 1987) used the same approach as described above for HCB and concluded that 450 µg/kg HCBD in the diet would be associated with a 1/1000 cancer risk in mink and Lerche et al. (2002) reported a water NOEC of 6.5 µg/L for fish. The EU EQS for HCBD has been set about an order of magnitude below those values at 55 µg/kg for prey and 100 ng/L annual average (600 ng/L maximum) for water.

HCBD does not occur naturally and previous contamination has been associated with industrial plants such as at Runcorn near Liverpool in the UK, where HCBD was found to seep into houses from an industrial landfill site causing health problems for some of the occupants (Staples et al., 2003) and near where it was also detected in marine water and biota back in the 1970s (Pearson and McConnell, 1975), albeit at fairly low levels (maximum water concentration of 30 ng/L and maximum fish concentration 0.4 µg/kg in flesh and 2 µg/kg in liver). High concentrations were found in eels from the river Rhine in 1993, with average concentrations exceeding the EQS of 55 µg/kg at about 10 % of sites (Heinisch et al., 2004). In the 1970s concentrations around 1 mg/kg were found in fish in the Ketelmeer a lake fed by the river Rhine in Holland, but in the adjoining Ijsselmeer the concentrations were almost 10 times lower while water concentrations at the same sites were then near the current EQS of 0.1 µg/l (Goldbach et al., 1976). The Environment Agency monitored sites along the Rivers Thames and Welland since 2006 and has failed to find HCBD in the water above the detection limit of 3 ng/L in any of the 136 measurements (table 1). No reports in the wider published literature on recent concentrations of HCBD in river water have been found and a recent paper from France failed to detect any HCBD in four species of fish from the river Rhone at a detection limit of about 10 ng/g dry weight which is 2-3 µg/kg ww (Miege et al., 2012), while a study from Belgium analyzed 20 eels and found a maximum of 12 µg/kg ww and a median of about 0.2 (Roose et al., 2003).

Mercury is a rare element, comprising only 0.08 ppm in the earth's crust on average, though local concentrations can be much higher (Jonasson and Boyle, 1971). It is one of only two elements that are liquid at room temperature and evaporates easily, even at relatively low temperatures, allowing it to spread widely via the atmosphere. Mercury has in the past been used for various chemical processes as well as in agriculture, but because of its known toxicity it has now been reduced, or replaced in most applications. However, as an element it cannot actually be completely removed from the environment only made more or less bioavailable. Mercury in metal ores, fossil fuels or deep sea sediments, for example, have low availability due to their location and/or chemical form, but when ores or fossil fuels are heated the mercury can enter the atmosphere and become much more available. Consequently some efforts are underway to remove unwanted (mainly metallic) mercury from circulation by depositing it in safe storage areas such as salt mines. Globally the main deliberate uses of mercury today are in small scale gold (and silver) mining, dentistry, as a catalyst in the production of polyvinylchloride (PVC) from coal and in the chloralkali industry. Of these, only dental amalgam, which contains about 50% mercury and the chloralkali industry remain important in the EU, accounting for about 2/3 of the total use with 90 and 190 t/a respectively in 2005 (European Commission - Directorate General for Environment, 2006). Important sources of mercury to the environment

include the burning of wood and fossil fuels, the primary production of other metals such as Fe, Cu, Pb, Zn from ores, and cement production, all releasing mercury contained as a trace contaminant in the raw materials. The latest estimates by the United Nations Environment Programme (UNEP) suggest that worldwide small scale gold mining was the most important source of mercury emissions to air in 2010, but for the EU goldmining is negligible and coal contributes about half of the total of 87.5 t/y (44.5-226) followed by metal and cement production with about one eighth each (UNEP, 2013). Significant amounts can also be released to air naturally when mercury containing ores are heated during a volcanic eruption, but overall anthropogenic sources greatly exceed natural geogenic processes (Driscoll et al., 2013 in press). In addition to the mercury released to air which reaches rivers by wet and dry deposition and from erosion of soils, where it has previously been deposited, some direct inputs into freshwater are relevant: UNEP (2013) estimates non-ferrous metal production and consumer product waste are responsible for around 90t/a each globally, but has not yet quantified some other potentially important sources such as PVC production.

In the environment, the inorganic forms of metallic Hg^0 and inorganic Hg^{2+} are readily transformed to methylmercury by microbial action. Methylmercury is highly toxic, especially to the developing nervous system, and it accumulates in the food web. Sandheinrich and Wiener (2011) reviewed the observed effects on fish experimentally exposed to methylmercury via food, or water expressed as the observed body burdens. At body burdens in the hundreds of µg/kg various negative effects including effects on survival, growth, and suppression of fertility were observed. When grayling eggs were exposed to methylmercury via the water for 10 days, those groups that had body burdens of 270 µg/kg, or over, as newly hatched fry still showed reduced feeding efficiency and competitive abilities as 3 year old adults (Fjeld et al., 1998). Field studies also found correlations between mercury concentrations and sex hormones, enzyme activities, histological changes, condition factor, gonadosomatic index, and hepatosomatic index at concentrations well below 1000 µg/kg (reviewed in Wiener et al., 2003). Sandheinrich and Wiener (2011) concluded that the threshold for negative effects on fish is between 300 and 700 µg/kg for whole body homogenates. From the data given in the same review, it can be estimated that the biomagnification factor of methylmercury between the contaminated food and the experimental fish is usually around 4, although none of the reviewed studies exposed fish for a full life cycle, so this may be an underestimate. Safe levels for mercury in fish in the diet of otters have been proposed between 100 µg/kg and 500 µg/kg (Boscher et al., 2010). These concerns help to explain the relatively low EU 20 µg/kg EQS for Hg in prey with the water limit being 50 ng/L annual average and 70 ng/L maximum. Mercury in fish has been extensively studied because

marine fish and other seafoods are the main dietary source of mercury for humans, which is estimated to be still high enough to reduce the intelligence quotient (IQ) of about 1/3 of babies born in the UK, which is about average for Europe (Bellanger et al., 2013). Many countries have set standards to protect human consumers from mercury in food - for example in the EU the limit for mercury in fish for human consumption is 500 µg/kg (1000 µg/kg for eel) (Commission Regulation (EC) No 1881/2006) and in the US 300 µg/kg are recommended (U.S. Environmental Protection Agency (US EPA), 2001), but apart from the EU only Canada (tissue residue quality guidelines for the protection of wildlife consumers of aquatic biota, http://ceqg-rcqe.ccme.ca/ accessed 20.4.2013) has a standard designed to protect fish eating animals at 33 µg/kg ww.

Environment Agency water monitoring at Caversham on the river Thames returned 78% non-detects between 2006 and 2012 with the highest recorded value being 28 ng/L (table 1), which is below both the maximum and annual average EQS for water. In a study of the R. Lee catchment, a highly impacted river of the Thames catchment, mean Hg values of 40 ng/L were recorded for the period of 1991-2000 (Snook and Whitehead, 2004) and the Europe-wide geochemistry survey (FOREGS project, Salminen et al., 2013) reported a median river bed-sediment concentration for Hg as 38 µg/kg. Mercury concentrations in freshwater fish have been monitored in many countries and species. The best datasets exist for eels, which often have higher contamination than other freshwater species from the same site (eg. Downs et al., 1999; Edwards et al., 1999; Noel et al., 2013; Yamaguchi et al., 2003). Noel et al. (2013) provided an overview for recent European monitoring data in several species of fish including roach and eel: For eel the overall range in concentrations was very large from about 10 to 800 µg/kg but most studies had average concentrations around 100-200 µg/kg, whereas in roach the concentrations were mostly between 50 and 100 µg/kg with the exception of some higher values in Slovakia and the Czech Republic. There are indications of a reduction in mercury contaminations of freshwater fish in the UK (eg. Downs et al., 1999) or elsewhere (eg. Lepom et al., 2012), but most measured concentrations remain clearly above the EQS of 20 µg/kg ww.

Polybrominated diphenyl ethers (PBDEs) have been proposed for additional biota EQS (European Commission, 2012). They were, until relatively recently, extensively used as flame retardants mainly in electronic equipment and polyurethane foams used in upholstery. Usually PBDEs are used as additive flame retardants meaning that they are not chemically bound to the product they are protecting. The so-called penta-mixes consisting mainly of congeners 99 (2,2',4,4',5-penta-BDE) and 47 (2,2',4,4'-Tetra-BDE) with smaller amounts of penta-BDE 100, hexa-BDEs 153 and 154 and Penta-BDE 85 were the most commonly used until they were banned in the EU under the

recast Restriction of Hazardous Substances Directive (RoHS, 2002/95/EC). Due to their high Log K_{ow}s (6.57 for penta-BDE, 8.35-8.9 for octa, and 9.97 for deca), very little is found dissolved in water with the majority being bound to the organic fraction of suspended and bed-sediments, or the lipid in aquatic organisms (Tlili et al., 2012; Wenning et al., 2011). Airborne particle transport is believed to be responsible for PBDEs being found in ice cores as far away as the arctic circle from the 1970's onward (Hermanson et al., 2010), but compared to HCB there is a much greater geographical variation of atmospheric concentrations with the UK being a European hotspot in samples from 2002, which was believed to be related to their high production and use there (Jaward et al., 2004) and in a survey of eels across Europe the UK sample also had the highest PBDE concentrations (Santillo et al., 2005). Few studies on the toxicity of PBDEs to aquatic wildlife exist, but Muirhead et al. (2005) found a clear reduction in fertility and condition factor in male fathead minnows exposed to BDE 47 contaminated food. Extrapolating from studies on the neurodevelopment in mice the EFSA Panel on Contaminants in the Food Chain (CONTAM) (2011) derived body burdens at which an effect might be expected in humans by calculating the $BMDL_{10}$ (bench mark dose, lower 95% confidence level for a 10% response) as 309 µg/kg for BDE-47; 12 µg/kg for BDE-99, 83 µg/kg for BDE-153 and 1,700 µg/kg for BDE-209. Fish take up PBDEs mainly through their food and since the chemical tends to be associated with sediments, bottom dwelling fish are often more contaminated than pelagic fish (Wenning et al., 2011). Tomy et al. (2004) reported biomagnification factors between 35 and 45 for the 6 PBDEs, which are in the proposed EQS, when juvenile lake trout were fed PBDE spiked food at high concentrations. The current legislation specifies an annual average water EQS of 0.5 ng/L for the sum of 6 commonly found PBDEs (congener numbers: 28,47,99,100,153,154), whereas the new proposals aim to lower this to 0.049 pg/L (annual average) and 0.14 ng/L maximum and add a biota EQS of 8.5 ng/kg ww. Law et al. (2008) reviewed PBDE concentrations in a variety of matrices including fish: Typical concentrations for European freshwater fish are from the hundreds of ng/kg to the low tens of µg/kg for the sum of 6 BDEs. Roosens et al. (2008) reported similar values when reviewing BDE 47 (which is typically about 3/4 of the sum of 6 BDEs) in eels, though samples taken from an industrialized region of Belgium were higher with an average of 77 µg/kg ww and from the data given in further Belgian study (Roosens et al., 2010) wet weight concentrations of the sum of 6 BDEs in eels can be estimated as having a median of 5 µg/kg in 2006 with a wide variation of concentrations (ca. 0.2-750 µg/kg ww). Recent European river water concentrations for the sum of 6 PBDE were reported below the current EQS of 0.5 ng/L at 0.37 ng/L in the Po (Italy), 0.3 ng/L in the Danube (Hungary) and 0.23 ng/L in the Meuse (Netherlands) rivers (Hanke et al., 2012) and 0.02-0.27 ng/L for the sum of 11 tri-hepta BDEs (including the 6 in

the EQS) in the Seine (France) (Tlili et al., 2012). In an inter-laboratory comparison exercise only 20% of participating laboratories were able to detect all 6 EQS PBDEs at the requisite limit of quantification (LOQ) of 30% of the current EQS (for the sum of 6 BDEs, therefore for each individual one the LOQ should be 5% of EQS) (Hanke et al., 2012).

2 Material and methods

2.1 Fish sampling

Fish were caught at several sites along the Rivers Thames, Kennet, Nene and Glen (table 2). Using data from the National River Flow Archive (NRFA), Landcover map 2000, and standard-period average annual rainfall (SAAR) for 1961-90 (summarized in the IRN/RACQUEL program developed by CEH: http://wlwater.ceh.ac.uk/racquel/ and in Marsh and Hannaford (2008)) the catchments can be characterized as follows: the Thames in southern UK has a catchment area of 9,948 km^2, a length of 255 km to the tidal limit, 14% of the catchment is taken up by settlements (classes: urban or suburban/rural developed), agriculture covers 36%, grassland 32% and woodland 16% and the catchment receives 700 mm annual rainfall. The Kennet is a 93 km long tributary of the Thames with a catchment area of 1144 km^2. The annual rainfall in this catchment is about 750 mm. The River Nene in eastern UK is 169 km long to the tidal limit and has a catchment area of 1,666 km^2 upstream of the lowest gauging station (36 km from the tidal limit). 53% of the catchment is taken up by agriculture with about 10% urban or rural settlements. The Glen is a 71 km long river in the same area with a catchment area of 213 km^2 mainly through agricultural land (70%) which is low lying and therefore known locally as "Holland". Only 5% is urban or rural settlements and 15% is grassland (see figure 1 for an outline, a detailed map of the sites is available through the journal website). All data is for the non-tidal parts of rivers.

Fish sampling was carried out by fish monitoring teams of the Environment Agency of England and Wales (EA) using either seine nets or electro-fishing by wading or from a boat depending on the depth of the river. The EA fish monitoring strategy is to catch all the fish in a stretch of river and record species, numbers, and lengths before releasing them back into the river. For the fish tissue archive the aim is to collect a sub-sample of 10 roach (*rutilus rutilus*) of approximately 15 cm length per year at each sampling site, though actual sizes and sometimes numbers varied depending on availability. In 2007 and 2008 additionally bleak (*alburnus alburnus*) were collected and samples of eels (*anguilla anguilla*) were also provided by the EA in 2007. The eels were in the yellow eel stage with most having very limited or no gonad development apart from two large males in each of the two sites which

had well developed gonads. The fish were killed using an overdose of 2-phenoxyethanol (ca. 4 ml in a 10 L bucket), weight and length recorded and then frozen on site in fluoro-ethylene-propylene (FEP) bags placed in a liquid nitrogen cooled dry shipper. On return to the laboratory the frozen fish were transferred to a -80°C freezer. Roach and in 2007 and 2008 also bleak were chosen because they are relatively common in UK rivers and rarely stocked. Although individual roach have been observed to move 10 km or more, many stay in a shorter stretch (Baade and Fredrich, 1998; Geeraerts et al., 2007) and in the Thames, Kennet, and Nene their movement is further restricted by locks creating barriers.

2.2 Sample preparation and analysis

For all samples from 2008 onwards the whole frozen fish were ground into a powder without defrosting them using a cryogrinder (SPEX SamplePrep 6850). The resulting frozen fish powder was divided into pre-cooled 20 ml glass scintillation vials and stored at -80°C until use. In the initial setup phase of the fish archive in 2007 the cryogrinder was not yet operational. Therefore the eels were cut into sections before freezing and one section was used for analysis and the roach and bleak were briefly defrosted and dorsally divided in half, with one half being analyzed for persistent organic chemicals and the other half returned to the -80°C freezer and later ground for analysis of metals. A few of the fish sampled in 2007 had the liver and gall bladder dissected out and the liver was analyzed for persistent organic pollutants separately from the remaining carcass and the bile for endocrine disruptors (Fenlon et al., 2010). Although concentrations of hydrophobic POPs tended to be higher in the liver than the rest of these fish, the difference largely disappeared when the results were lipid normalized. Non-detects were more frequent in liver samples than whole fish, because due to the small size of the livers the amount extracted had to be reduced – sometimes more than 10 fold -increasing the detection limits, so the study subsequently focused on whole body extractions.

To test whether the cryogrinding process introduces metal contamination of the sample, trout fillets were purchased from a local supermarket. Half were skinned and the other half retained the skin. They were then cut into approximately 2 cm strips with alternating strips being used in the cryogrinder and left unground. Since no difference in mercury concentration was found, we present the averages of the respective ground and un-ground fish.

For the organic analysis, around 5 g of the whole fish homogenate was mixed with 30 g sodium sulphate to remove the water, then recovery standards ($^{13}C_{12}$ PCB mix: 28, 52, 101, 138, 153, 180 and PBDE mix 51, 128, 190) were

added and the mixture extracted over night with dichloromethane (DCM) in a soxhlet apparatus. Procedural blanks without the fish homogenate were run with every batch. A portion of the extract was used to determine the lipid content, by weighing the oily residue after the DCM had evaporated and the remaining extract was used for the determination of persistent organic contaminants: The DCM was evaporated in a vacuum rotary evaporator and replaced with 10 ml hexane, which was reduced to about 1 ml. This was added to a glass column with 11 g acidified silica (200 ml silica baked at 450°C and acidified with 25 ml concentrated sulfuric acid) and eluted with hexane as a first clean up step, which removes the fats. Then the sample was passed through a gel permeation chromatography column with 50:50 Hexane:DCM and only the fraction from 17 to 51 ml collected as second clean up step to remove molecules outside the size range of interest. The solvent was then replaced with hexane and the sample added to 25 µl internal standards (PCB 30, ^{13}C-PCB141, ^{13}C-PCB208, BDE69, BDE181) in dodecane, before evaporating the hexane, so that the whole sample was contained in the 25 µl dodecane. The extracts were analyzed by gas chromatography – mass spectrometry (GCMS, Thermo Trace, electron impact mode, single ion monitoring, source temperature 250°C, splitless injection. Columns: 50 m CPSil8, 0.25mm ID, 0.12 µm film (Varian) for HCB and HCBD and 30 m, DB-5, 0.25 µm ID, 0.1 µm film (J&W Scientific) for PBDEs). The instrument limit of detection (LOD), defined as the lowest observable standard was between 1 and 6.25 pg/µl for the analyzed chemicals, which is equivalent to 5-31 ng/kg for a 5 g sample.

For mercury 1-2.5 g wet weight was weighed into a PTFE vial, digested in a microwave digester (MARSXpress, CEM) with 10 ml ultrapure nitric acid (Baker, Ultrex II), then made up with ultrapure water (>18 MΩ/cm) to 25 ml before analyzing using a Perkin Elmer Elan DRC II inductively coupled plasma mass spectrometer (ICPMS) instrument. Certified reference materials (DORM-3 and additionally DOLT-4 for later batches, both from National Research Council, Canada) were analyzed alongside each batch and measured at 92-140% of the published mercury values. The LOQ was about 10 µg/kg ww for a 2 g sample. The age of roach and bleak were estimated from their lengths using reference data for average length-age relationships in UK rivers (Britton, 2007). In order to compare with the EU EQSs all results are given on a wet weight basis.

2.3 Statistics

All Statistical analyses were performed on log transformed data. For mercury enough data was available to perform an analysis of co-variance (ANCOVA) with weight or estimated age as the co-variant. For the organic chemicals HCB and PBDEs analysis of variance (ANOVA) was performed. This was followed up with F-tests for

equal variance and pair-wise Student's t-tests assuming equal or unequal variance as appropriate. For all statistical analyses a significance value of α=5% was used.

3 Results

For HCB, none of the samples analyzed exceeded the EQS of 10 µg/kg (table 2, figure 2). There were species differences with the eels tending to have higher concentrations overall than bleak and roach and higher concentrations than bleak from the same site, although with a 14% probability of arising by chance alone this difference was not significant at the 5% level. This larger burden in the eels may be due to the greater age, or lipid content of these fish and indeed the differences between species or between individuals from the same sampling occasion were reduced when the data was lipid normalized (see table 3, showing that the relative standard deviations are usually lower for lipid normalized data than for wet weight). Except for the Sunbury-Molesey eels, which ranged from 0.05 to 6.4 µg/kg, the variation in HCB burdens between fish collected from the same reach was always within one order of magnitude, with more than 95% of the non-tidal samples from all species and 60% of the tidal eels less than a quarter of the EQS of 10 µg/kg (figure 2).

HCBD was detectable in only 12 of the 38 samples examined and all values were below 0.2 µg/kg (table 3).

For mercury, there was no difference between the values returned by the ground and un-ground samples of trout fillets, showing that the grinding process did not contaminate the samples with additional mercury. For the wild fish samples, the vast majority (79%) measured higher Hg levels than the EQS of 20 µg/kg (figure 3), but all were about an order of magnitude below the limit for human consumption of 500 µg/kg (1000 µg/kg for eel) (Commission Regulation (EC) No 1881/2006). With the exception of the Nene, Cogenoe site, the variation in concentration between fish from the same location was less than 3-fold and the overall range of values was also relatively tight ranging from 6 to 68 µg/kg. Only at the two most upstream sites in the Thames catchment namely the Kennet tributary and the most upstream site of the Thames itself at Castle Eaton were the majority of analyzed fish below the EQS. The mercury contamination was related to fish weight with an overall R^2 of more than 0.2 for either roach or bleak samples (linear regression on log transformed data). For the roach the relationship between weight and mercury contamination became clearer with R^2's above 0.3 when the Kennet and Castle Eaton sites were calculated separately from the rest, confirming that their lower mercury contamination compared to the other sites is not an artefact due to size (figure 4), ANCOVA analysis confirmed that the correlation with weight was significant at α=5%. Bleak were only analyzed at the more downstream sites in the Thames and their mercury

contamination also increased with weight, having generally a higher mercury contamination than roach of the same weight (figure 4). To test whether this difference between roach and bleak is explained by bleak being smaller than roach of the same age (or vice versa a bleak being older than a roach of the same size) the known lengths of the individuals were converted into estimated ages using reference data for the UK for those species (Britton, 2007), which reduced the difference between the two species (figure 5).

The proposed PBDE flame retardants EQS of 0.0085 µg/kg for the sum of congeners 28,47,99,100,153,154 was exceeded in all the samples analyzed, with the highest concentrations being found in the river Nene (5.3-44 µg/kg) and the lowest concentrations in the Glen (2.0-4.6 µg/kg) (figure 6).

4 Discusssion

The whole body (minus liver and gall bladder in a few 2007 samples) HCB concentration in this study ranged from undetectable to about 6 µg/kg with the higher values mainly found in the estuary eels, but the differences between species or site were mostly not statistically significant (see figure 2). There was no evidence for an increase of HCB concentration with fish size. In fact, the largest roach in this study were caught in the relatively rural River Glen and those had the lowest contamination both for HCB and PBDEs, reflecting perhaps the low impact of industrial sources or the type of agriculture in that area. The HCB values found in this study are comparable to recent studies of bass in Chicago of around 3 µg/kg (Lozano et al., 2012), and eels from Scotland where most had < 3 µg/kg HCB and the maximum concentration was 7.2 µg/kg (Macgregor et al., 2010). Higher concentrations of HCB have been found along the river Rhine, where eels of more than 10% lipid content typically contained 20-200 µg/kg and occasionally more than 1000 µg/kg in 1993 (Heinisch et al., 2004), and where German annual monitoring of bream reported values which were relatively constant between 5 and 15 µg/kg since 1995. In the Danube, which has been monitored since 2004, the bream data were similar to the data in the current study at 1-4 µg/kg and in the River Elbe the contamination was higher at about 10-30 µg/kg since 2004, but had reduced 5-10 fold since it peaked in the 1990s at 80-150 µg/kg (http://www.umweltprobenbank.de/en/documents, accessed 21/8/2012). Eels caught in 2004 in the Netherlands (de Boer et al., 2010) had generally somewhat higher HCB contamination than those in the current study with seven of nine sites estimated to be higher than the EQS (we estimated the average wet weight concentrations from lipid normalized data and average lipid contents as 5-30 µg/kg).

HCBD does not seem likely to be of widespread concern in the English rivers analyzed here as it was non-detectable in the majority of samples and even where it was detected, it was more than two orders of magnitude below the EQS of 55 µg/kg. In a recent survey of eels in Scotland (Macgregor et al., 2010), HCBD was only detected in one of 150 samples at detection limits of either 1 or 3 µg/kg and the authors of a recent French study also failed to detect any HCBD at a detection limit of 2-3 µg/kg ww in fish and consequently questioned the need for a European EQS for this substance (Miege et al., 2012) and (Roose et al., 2003) found a maximum of 12 µg/kg in eel from an industrial area of Belgium. The river Rhine with its associated chemical industry again appears more contaminated -at least in the past- where concentrations over 100 µg/kg were measured in some eels in 1993 (Heinisch et al., 2004).

The mercury values measured in the present study while often exceeding the 20 µg/kg biota EQS, are much lower than they have been 20 or 30 years ago in England when average levels around 100 µg/kg were common in many species and individual fish often exceeded the food safety standards of 1000 µg/kg for eel and 500 µg/kg for other fish (Barak and Mason, 1990a; Barak and Mason, 1990b; Barak and Mason, 1990c). The mercury concentrations in the current study were almost a factor 10 lower than those found in Germany in 8-12 year old bream where the muscle tissue Hg values were mostly around 200 µg/kg in the most recent (2009) samples (Lepom et al., 2012), but very comparable to values for roach from the upper Thames where in 1995 the average muscle Hg concentration was 55 µg/kg in fairly large individuals (130 g average) (Yamaguchi et al., 2003) and from East Anglia (UK), where the average muscle Hg concentration in 1996 was about 27 µg/kg in roach (average weight about 110 g) (Downs et al., 1999). Mercury may be higher in muscle samples than in whole body homogenates, but Goldstein et al. (1996) found this difference to be usually less than a factor 2. Mercury concentrations tend to increase with age (Boscher et al., 2010) and trophic level, so the lower values found for the relatively small roach and bleak collected in this study compared to the much larger bream in Germany may reflect this. The values measured in the present study are indeed in the same range as those measured in 2007 in whole body homogenates of chub and barbel of a similar size from Luxembourg: their concentrations ranged from 10-68 µg/kg for the sites where all analyzed fish were small (27-120 g), whereas several hundred µg/kg were found in larger (1-2 kg) chub, barbel and eels (Boscher et al., 2010). The data from the present study showed a general increase of mercury concentration with size or estimated age of the individuals for both roach and bleak samples and a generally higher contamination of bleak compared to roach of the same weight or age. The observed differences between sites could largely be explained by the different sizes of fish, with the exception of the two most upstream sites in the Thames catchment where roach

had lower mercury concentrations for their weight or estimated age than at the other sites (figures 4 and 5). Bleak were not sampled at those upstream sites. All the differences between groups and all the relationships with weight or estimated age plotted in figures 4 and 5 are significant at the 5% level (ANCOVA). Differences between species and the increase of Hg concentration with size/age, demonstrate the importance of choice of species and size. In the current version of the legislation there is no prescription on size, age or species of sampled "prey", but Lepom et al. (2012) found that the concentration of mercury in zebra mussels was approximately 25 times lower than the concentration in bream caught in the same year and stretch of river. Zebra mussel have only about 5% dry weight compared to about 25% in fish, so part of the difference can be explained by their higher water content, but even allowing for dry weight a five-fold difference remains. To be able to compare between different sites and times, fish of the same species, age, condition factor etc. would ideally be collected. Practically this is not possible, not least because there are no species that would be found across all water bodies that could, or should, be monitored. However, if species are chosen which are similar in terms of size, age and life style, it should be possible to make reasonable comparisons across space and time.

For PBDE, all the fish sampled exceeded the proposed biota standard of 0.085 µg/kg (figure 5) with an overall range of 2 to 44 µg/kg for the sum of 6 BDE or 1.5 – 31 µg/kg for the dominant congener BDE 47, which is broadly similar to other recent European data, though the values for the Nene samples are among the higher ones reported. For example, a recent survey of five species at six sites in the Czech Republic found average concentrations between 0.2 and 18 µg/kg ww for the sum of 6 BDEs (Pulkrabova et al., 2007), while Miege et al. (2012) reported a range of 4.5-183 µg/kg dry weight (average 50) for fish in the Rhone (France), which translates to approximately 1-42 µg/kg wet weight. In the current study PBDE (sum of 6) concentrations were highly correlated with HCB concentrations for those individuals where both chemicals where measured ($p < 0.1\%$, or even $<0.01\%$ if the highest HCB outlier is removed, for log-linear regression), which is to be expected as both are widespread hydrophobic persistent organic pollutants, but neither of those two organic pollutants was significantly correlated with mercury. Using data from the European Food Safety Agency (EFSA) which estimated threshold body burdens at which humans may experience negative effects (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011) and applying those to fish, the lowest body burden at which an effect might be expected would be 12 µg/kg if all the PBDE was BDE 99. About half of the samples analyzed exceeded this value in the sum of the six monitored PBDEs but none exceeded it for BDE 99 alone. The largest contributor was always BDE 47, for which EFSA suggested a threshold of 309 µg/kg. This suggests, that these English fish were not in immediate

danger of harm from individual, or collective PBDEs. Indeed, in developing the proposed PBDE EQSs the authors of the dossier on PBDEs (European Commission, 2011) thought 44.4 µg/kg for the sum of 6 BDEs sufficient to protect wildlife predators (a value which was only reached in one individual analyzed), but calculated the much lower value of 0.085 µg/kg to protect human consumers based on observed effects of BDE 99 on rats and including very large safety factors. To ensure this low concentration in fish requires the very low proposed water EQS of 0.049 pg/L. However, the same dossier suggests a drinking water standard equal to the current surface water standard of 0.5 ng/L. Roosens et al. (2010) quotes reference dose values (RfD) of 100 ng/kg/day for the intake that would be harmful to humans for either BDE47 or BDE99, therefore a 70 kg human consuming 7 µg BDE47 and/or BDE99 every day might experience harmful effects. With the proposed EQS of 0.085 µg/kg ww that would equate to a daily fish consumption of 82 kg! Therefore we would agree that at levels above 44 µg/kg for the sum of 6 BDEs, there may be a concern for higher predators including humans, considering the biomagnification and that the individual congeners are likely to act in an additive fashion, but we find it difficult to understand the rationale for imposing an EQS as low as that proposed.

Above, we have shown the application of a fish tissue archive for monitoring the three current and one of the proposed EU biota standards under the Water Frame Work Directive and we believe that building up such an archive can be of wider use. In that context we considered the question whether bio-monitoring of wild fish is a sensible way to monitor contamination by hydrophobic/bioaccumulative contaminants.

When monitoring pollution of a water body, several approaches can be taken. The first and most common one is to take **water samples** at regular intervals, but the instantaneous concentrations of any chemical can fluctuate wildly due principally to flow rates, and therefore dilution, varying by several orders of magnitude throughout the year (Johnson, 2010). Furthermore water concentrations of many chemicals of interest are often present only at very low concentrations which can be a problem with precision and repeatability between laboratories (Hanke et al., 2012). For example, for mercury in monthly water surveys of the Thames at Caversham (an area covered by this survey), about 70% of values were below the detection limit of 0.01 µg/L since the method became sensitive enough to measure at that level in 1994, and there was no exceedance of the water EQS of 0.07 µg/L (maximum) and 0.05 µg/L (annual average) over that period (data provided by Environment Agency from the WIMS database, summarized for 2006 onwards in table 1) and yet mercury was detectable above the biota EQS in all fish samples from the same stretch of the river (table 2 and figure 3). The example in this paper for mercury shows that largely

non-detectable water concentrations may still give rise to tissue concentrations that could be of concern for top predators (tables 1-2, and figure 3). Trends of a chemical in biota may also be substantially different than those in water, for example Mathews et al. (2013) found little or no change in fish tissue concentrations of mercury in a highly contaminated stream over a 20 year period despite a five-fold decrease in the concentration in the water during the same time.

To avoid the short term fluctuation and low concentrations in water samples, **passive samplers** have been developed. Different types are optimized for different groups of chemicals and new ones are constantly developed. These are typically left in the water for a few weeks and in that time accumulate chemicals from the water. From the point of view of protecting wildlife, this approach, while being better than water grab samples, still suffers from the drawbacks that the exposure period is relatively short and uptake from food or sediment - either directly or via the food web - is not taken into account. When trying to back calculate the water concentration of HCB or PCBs from passive samplers or caged fish, quite different values were arrived at for the same stretch of river (Verweij et al., 2004). Whilst it could be argued over which were 'right' there can be no argument over which were more relevant for wildlife. From the point of view of long term monitoring of trends, there is the additional worry as to whether the chosen type of passive sampler will still be available many years into the future.

Another approach is in **active biomonitoring** for example with caged fish (Besse et al., 2012; Verweij et al., 2004), allowing them to accumulate chemicals both from the water (bioconcentration) and the food web (biomagnification). Fish can also be monitored for relatively polar contaminants, such as endocrine disrupting compounds, for example through examining the contents of their gall bladders (Fenlon et al., 2010). This is obviously more realistic with regards to protecting wildlife than the other approaches described above. Additionally, caged fish have the advantage over wild ones that factors such as size, species, sex etc. can be tightly controlled making it easier to compare different sites or times. However, a disadvantage is that in general the fish cannot be left for more than a few weeks because of problems with mortalities. Also, the cage severely restricts their opportunities to hunt for prey and often the stress associated with being in a cage prevents fish from feeding normally. If the fish are fed, then their food source must also be tested for all the chemicals of concern to check for contamination.

Wild fish and other biota accumulate chemicals from food and water over their whole lifetime. That way an indication is obtained of average concentration over several years and levels of pollutants are often much higher in fish or other organisms than in the surrounding water, making them easier to measure despite the more complicated

matrix . A practical issue is the occasional absence of wild fish of the selected species from the reach on the day of study and the possibility of movements due to migration or stocking confounding the results. In England and Wales the EA monitor fish every year in most of the major river basins, thus, a good database on fish abundance at different river reaches is available. This can help select locations where fish are likely to be found and also where removing a small sub-set of fish is sustainable. On the basis of this information, a fish monitoring exercise can be sustained. Stocking can be a problem, but unless the fish have been stocked very shortly before they are captured or they originated from a much more polluted site (which in the case of fish farms is unlikely), their chemical pollution would still be mainly influenced by the water in which they were captured. Importantly, if the reason for monitoring a pollutant is to protect wildlife from its effects then it is not actually the aqueous, or even the sediment concentration but the tissue concentration in the prey (whether native or stocked) that is most relevant.

When "prey" is monitored the choice of species and tissue sample is important. Most non-human predators would eat the whole of their prey and a main reason for monitoring and, if possible reducing, the contamination of wildlife is to protect predators from secondary poisoning. There is however an argument for removing the gut content on the basis that it contains largely non-digestible matter, which would remain non-digestible in a predator. Therefore, including the gut content could lead to an overestimate for some chemicals if the concentration is high compared to the rest of the body. In this case the whole-body homogenate could be seen as a worst-case scenario and exceedances of EQS could trigger a follow up investigation, which could look at gut contents separately from the actual body of the animals. On the other hand, chemicals may be found at lower concentration in the essentially non-digestible gut contents which would lead to an underestimate of the amount available to a predator. At least in the case of fish, this does not seem to be a large problem as the gut content is only a small proportion of the total weight of a fish. In many cases the approach is taken to monitor the organs that accumulate a chemical most or where the toxic effects are expected to be strongest. Which organ is the most contaminated and/or the most susceptible depends however on the chemical; for example, methylmercury tends to accumulate in muscle tissue more than in the liver (Wiener et al., 2003) while the opposite is true for hydrophobic organic chemicals (eg. Teil et al., 2012). Lastly, the use of whole body homogenates allows for large enough sample sizes to allow multiple analyses even from relatively small individuals, without having to resort to composite samples. We have chosen to focus mainly on roach but clearly other species could and should be considered too. In particular eels have a lot of advantages in terms of monitoring (Belpaire and Goemans, 2007), because they spend a long time in the same river during which time they don't spawn which might otherwise periodically reduce the body burden of some chemicals,

they have a high lipid content increasing the capacity to accumulate hydrophobic substances, and they are closely associated with the sediments, where much of the pollution is located. For these and other reasons, there is a larger body of knowledge on eel pollution than on any other freshwater species. However, given that European eels are now classified as a critically endangered species (http://www.iucnredlist.org/ accessed 1.5.2013), and that numbers in the UK in general tend to be lower further away from the south and east coast (Ibbotson et al., 2002), we would be reluctant to advocate the regular removal of significant numbers.

Ideally, a range of species from different trophic levels and/or a range of other samples such as water and sediment would be monitored to allow for temporal and spatial trends to be observed even when they differ between species (Bhavsar et al., 2010) or media and to test for the impact of factors such as sex differences, age, home range etc., but this has to be balanced with the expense of time and money involved and the need to limit the impact on the studied ecosystems from removing too many individuals. We have chosen roach as a relatively common species where sufficient numbers are present at most sites to allow for removal of usually 10 individuals without negative impact on the populations.

5 Conclusions

This exercise indicates that monitoring of rivers for hydrophobic, or bioaccumulative contaminants, such as those found in current and proposed EU Directives, using wild pelagic fish is both feasible and practical. Regarding the original hypotheses: That none of the chemicals with biota EQS proposed by the EU would be exceeded in any fish caught in English rivers has been falsified for Hg and PBDEs. Whether concentrations would differ appreciably between species, has not been conclusively proven in each case: eels had a higher HCB concentration than bleak from the same site, but this difference was not significant at the 5% level, but there was overall a significant (ANCOVA, α= 5%) difference between bleak and roach for Hg once the Hg increase in either species with weight or age had been taken into account. That contamination would be evenly spread with no differences between sites has also been falsified with both HCB and PBDEs in roach being lower in the samples from the Glen than the Nene and Thames and mercury contamination in roach, once adjusted for weight, being lower in the two most upstream sites in the Thames catchment than the other investigated sites.

Both spatial and temporal trends are likely to emerge more clearly as the number of samples grows.

It should be noted that whilst many fish exceeded the Hg EQS, meaning that they might be problematic to predators living mainly on a diet of fish, they were about an order of magnitude below levels of concern for human

consumption and, whilst all the fish analyzed exceeded the proposed low EQS for PBDEs, these concentrations, to our knowledge, would not be toxic either to fish themselves, or their immediate predators.

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Tables

Table 1 European Union environmental quality standards for inland surface waters (Directive 2008/105/EC, 2008), considered in this paper (in brackets proposed changes, (European Commission, 2012)) and recent water quality monitoring data.

substance	Water AA[b] EQS [µg/L]	Water max. EQS [µg/L]	Biota (fish) EQS [µg/Kg fw]	measured water conc. 2006-2012 [µg/L][a]		
				Nene, Thrapston	Thames, Caversham (km 162)	Thames estuary, Woolwich (km 297)
HCB	0.01[c] (none[d])	0.05 (0.05)	10 (10)		97% < 0.001, max 0.002 (n=30)	all <0.001 (n=76)
HCBD	0.1[c] (none[d])	0.6 (0.6)	55 (55)		100% <0.003 (n=30)	all <0.003 (n=76)
mercury	0.05[c] (none[d])	0.07 (0.07)	20 (20)	100% <0.01 (n=8)	78% <0.01, max 0.028 (n=46)	72% < 0.01, max 0.272 (n=76)
PBDE[e]	0.0005 ($4.9*10^{-8}$)	not applicable (0.14)	none (0.0085)		0.0004, 0.0010 (n=2)[f]	

[a] courtesy of Environment Agency of England and Wales from water quality monitoring in WIMS database
[b] annual average
[c] to be set stricter if biota is not used
[d] biota (fish) standard to replace the annual average
[e] sum of congeners #28,47,99,100,153,154
[f] one-off measurements in 2011 at Shepperton (km 234) and Sunbury (km 239), congeners below det. limit (0.0006 µg/L) are estimated at half detection limit

Table 2 Site information and summary of results

river	site name	site accronym	dist from source [km]	year	species	HCB (µg/kg fw) mean	HCB (µg/kg fw) RSD	(ng/g lipid) mean	(ng/g lipid) RSD	n	HCBD mean	n	mercury (µg/Kg fw) mean	mercury (µg/Kg fw) RSD	n	PBDE[a] (µg/Kg fw) mean	PBDE[a] (µg/Kg fw) RSD	(ng/g lipid) mean	(ng/g lipid) RSD	n
Glen	Pinchbeck West	Pb W	53	2009	roach	0.21	11%	8.6	18%	4			40.2	20%	5	2.98	33%	130	59%	5
Nene	Cogenoe	Cogn	40	2008	roach	0.92	39%	26.9	18%	5	<0.2	5	19.7	37%	9	22.75	66%	650	57%	5
	Thrapston	Thra	73	2008	roach	0.91	19%	14.3	8%	5	<0.2	5	27.0	42%	10	29.93	30%	469	24%	5
	Oundle	Ound	90	2008	roach						<0.2	9	43.1	31%	9					
Kennet	North Croft-West Mills	NCr-WM	58	2011	roach								20.6	30%	9					
Thames	Castle Eaton	C. Eaton	43	2011	roach								16.9	32%	10					
	Caversham-Sonning	Cav-Son	162-166	2008	roach	1.82	84%	26.1	39%	5			31.2	21%	10	6.06	26%	108	53%	5
					bleak						<0.2	2	43.4	26%	13					
	Temple-Marlow	Tmp-Marl	187-190	2007	roach								39.4	11%	5	9.34	36%	312	28%	4
					bleak								29.8	22%	5					
	Bray-Boveney	Bray-Bov	203-209	2008	roach						<0.2	8	25.0	18%	3					
					bleak						<0.2	1	34.7		1					
				2009	roach								26.8	16%	5					
	Old Windsor-Bell	OW-Bell	216-223	2007	roach	0.33	58%	7.0	61%	5(4)[b]			35.0	36%	5	6.34	19%	129	14%	5(4)[c]
					bleak								49.3	21%	5					
	Sunbury-Molesey	Sun-Mol	239-243	2007	bleak	1.04	34%	13.1	30%	9			29.2	39%	10	14.99	22%	187	10%	9
					eel[d]	1.87	90%	20.7	56%	11										
	Molesey-Kingston	Mol-King	243-246	2008	roach						<0.2	8	23.9	28%	10					
	estuary: Woolwich	Woolw	297	2007	eel[d]	2.49	64%	15.3	39%	24										

[a] sum of congeners #28,47,99,100,153,154
[b] lipid content was only available for 4 samples, the RSD for µg/kg fresh weight for the same 4 is 67%
[c] lipid content was only available for 4 samples, the RSD for µg/kg fresh weight for the same 4 is 14%
[d] body section

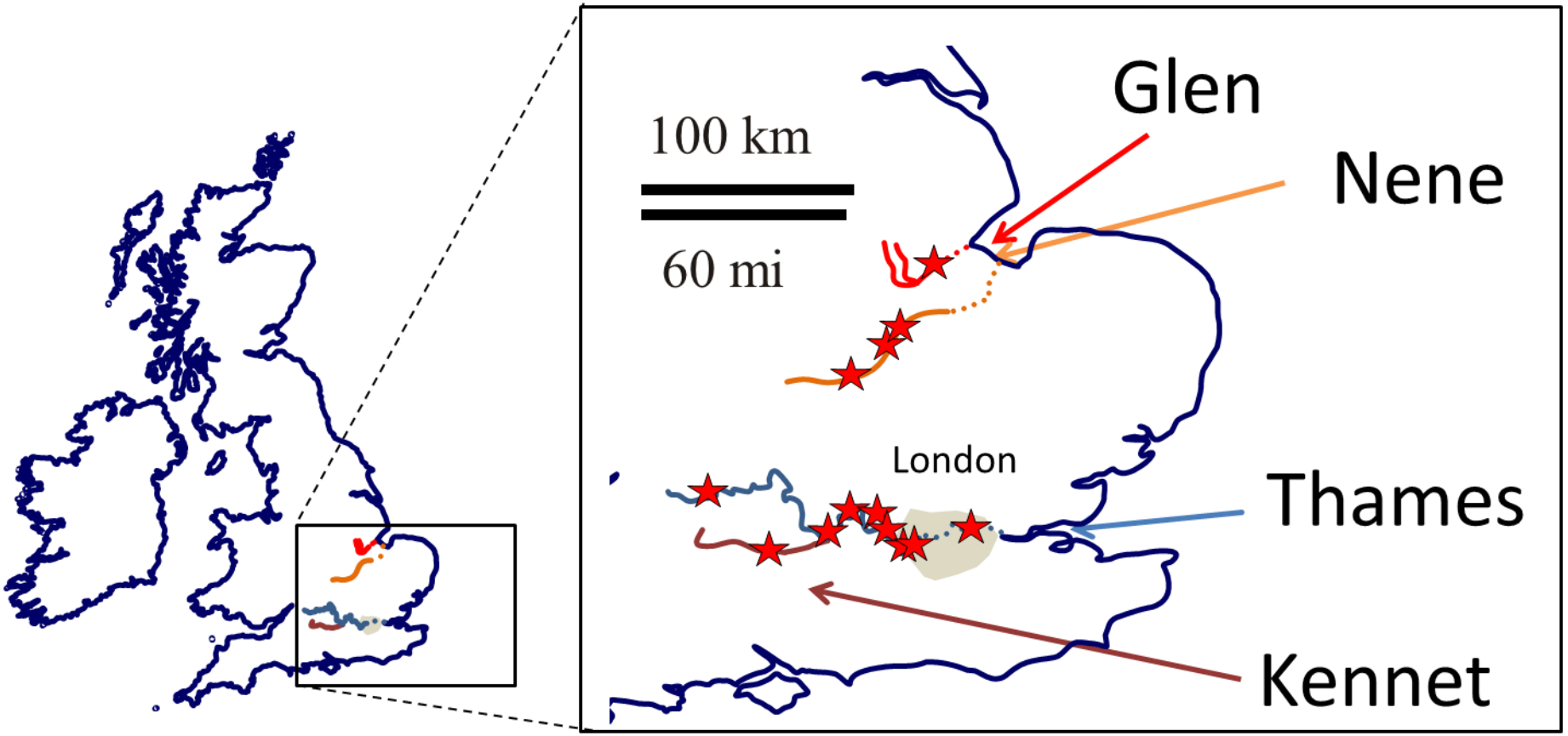


Figure 1: Location of rivers in the UK. The dotted lines represent the tidal area. Outline © d-maps.com

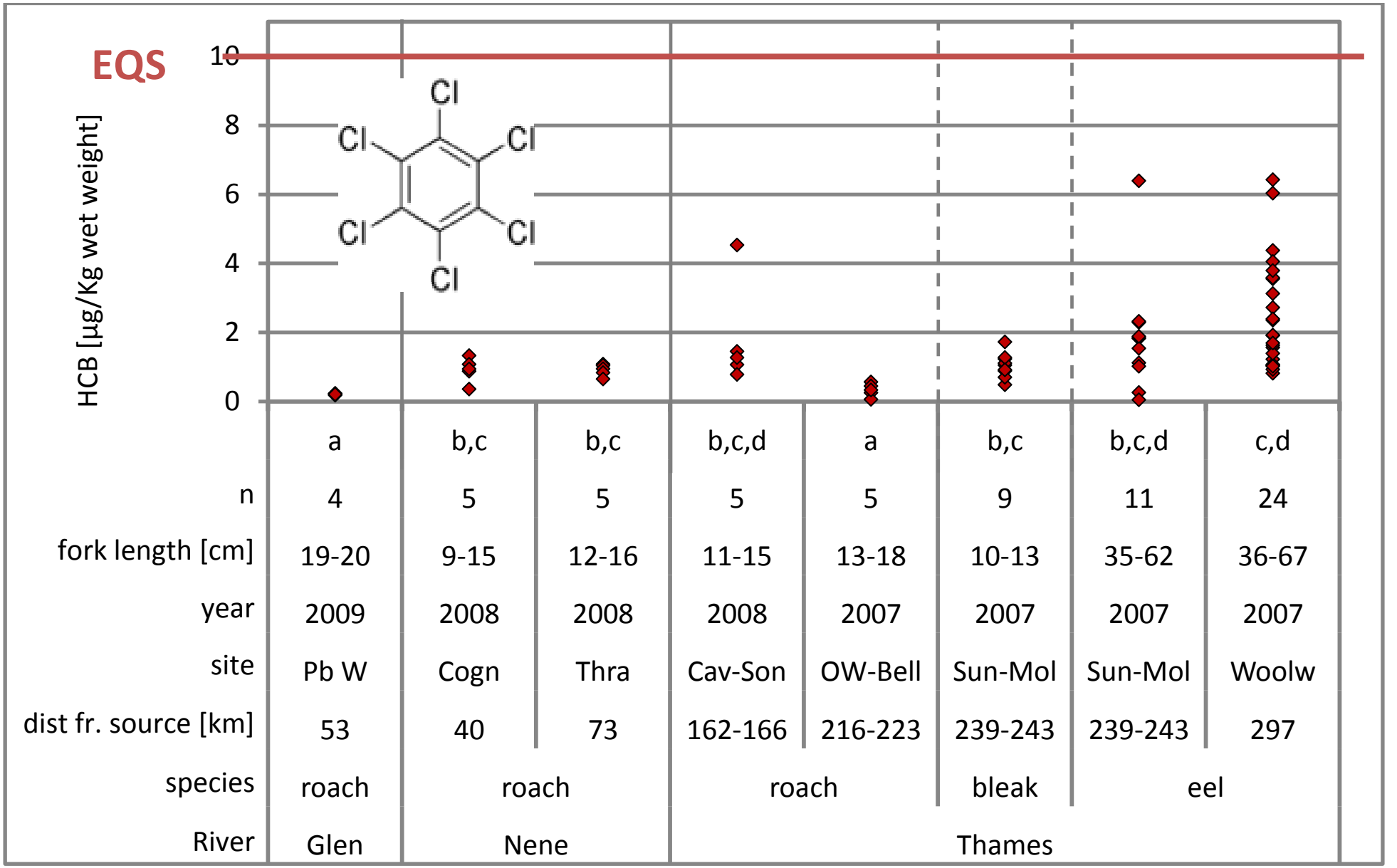


Figure 2: HCB concentrations in fish samples from English rivers (the symbols represent values for individual fish, site acronyms are explained in table 2). Different letters signify statistically significant differences at α=5% between groups in ANOVA and t-tests on the log-transformed concentration data. The thick red line illustrates the environmental quality standard for fish: 10 µg/Kg.

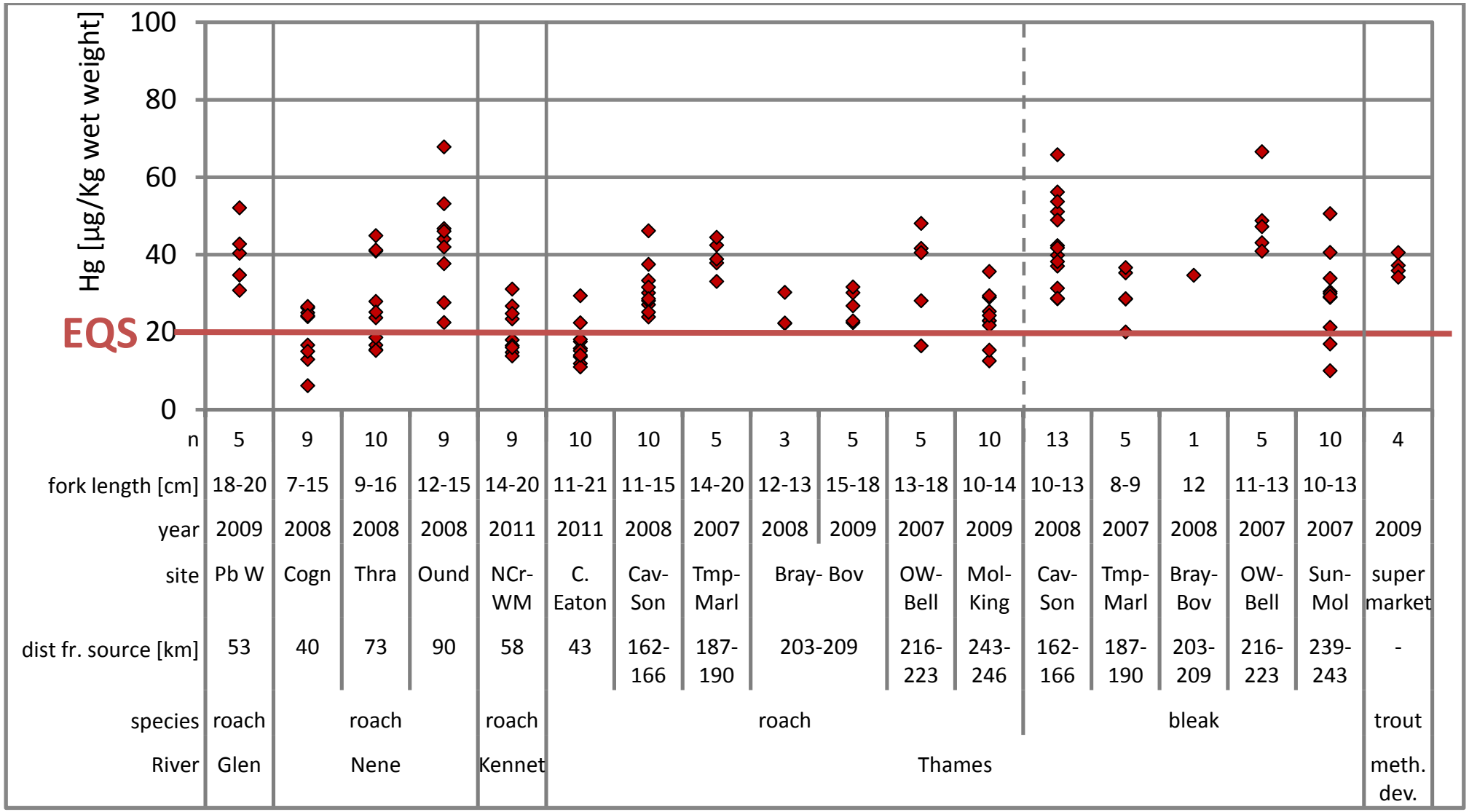


Figure 3 Mercury concentration in fish samples from rivers in England. The store bought trout fillets on the right were used for method development (site acronyms are explained in table 2). The thick red line represents the environmental quality standard for fish: 20 µg/Kg.

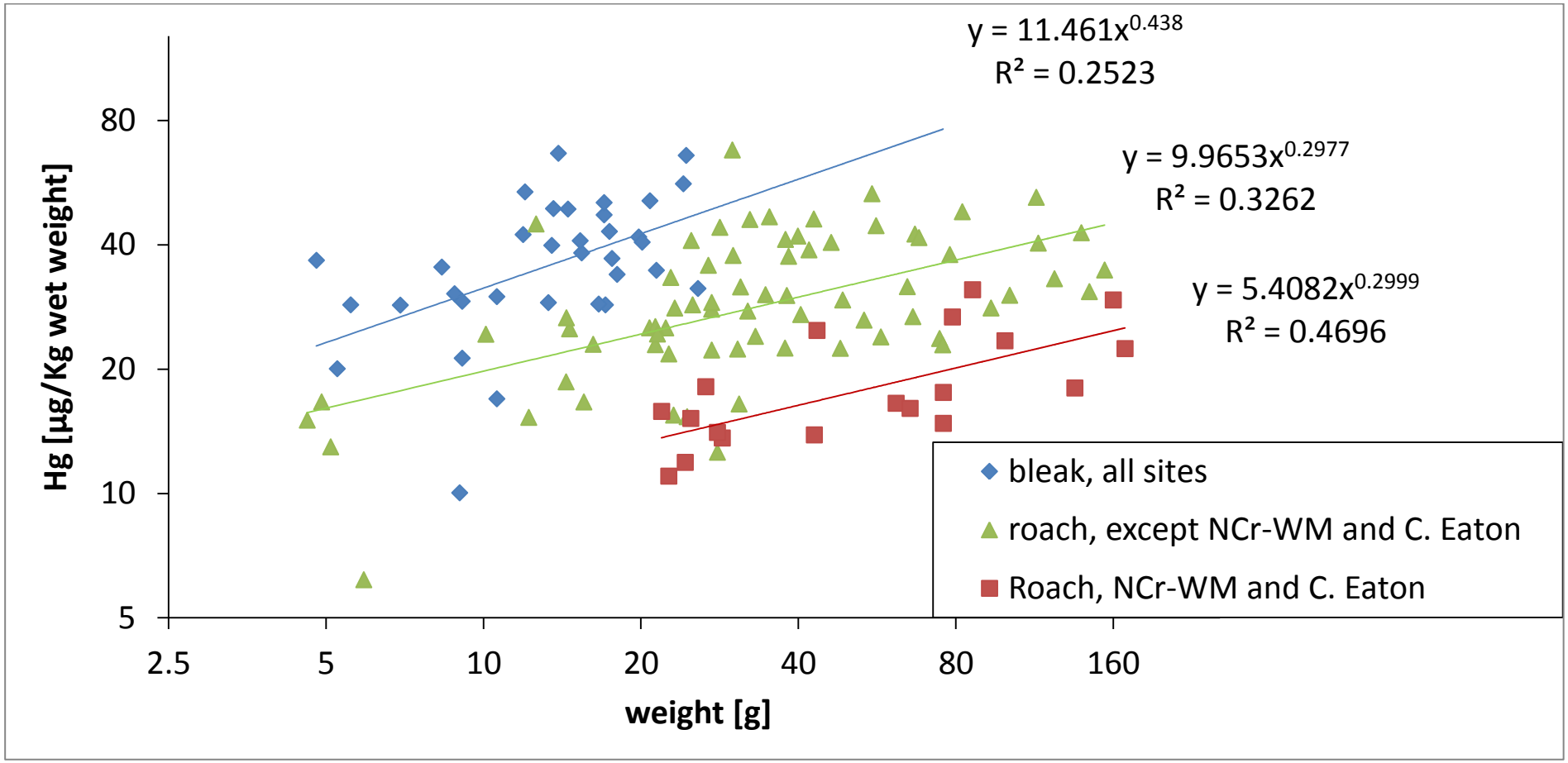


Figure 4 Mercury contamination vs weight for roach and bleak (log scale). Bleak were only sampled at downstream Thames sites, roach were divided into those from the most upstream sites in the Thames catchment: NCr-WM and C. Eaton, and the rest. Site acronyms are explained in table 2.

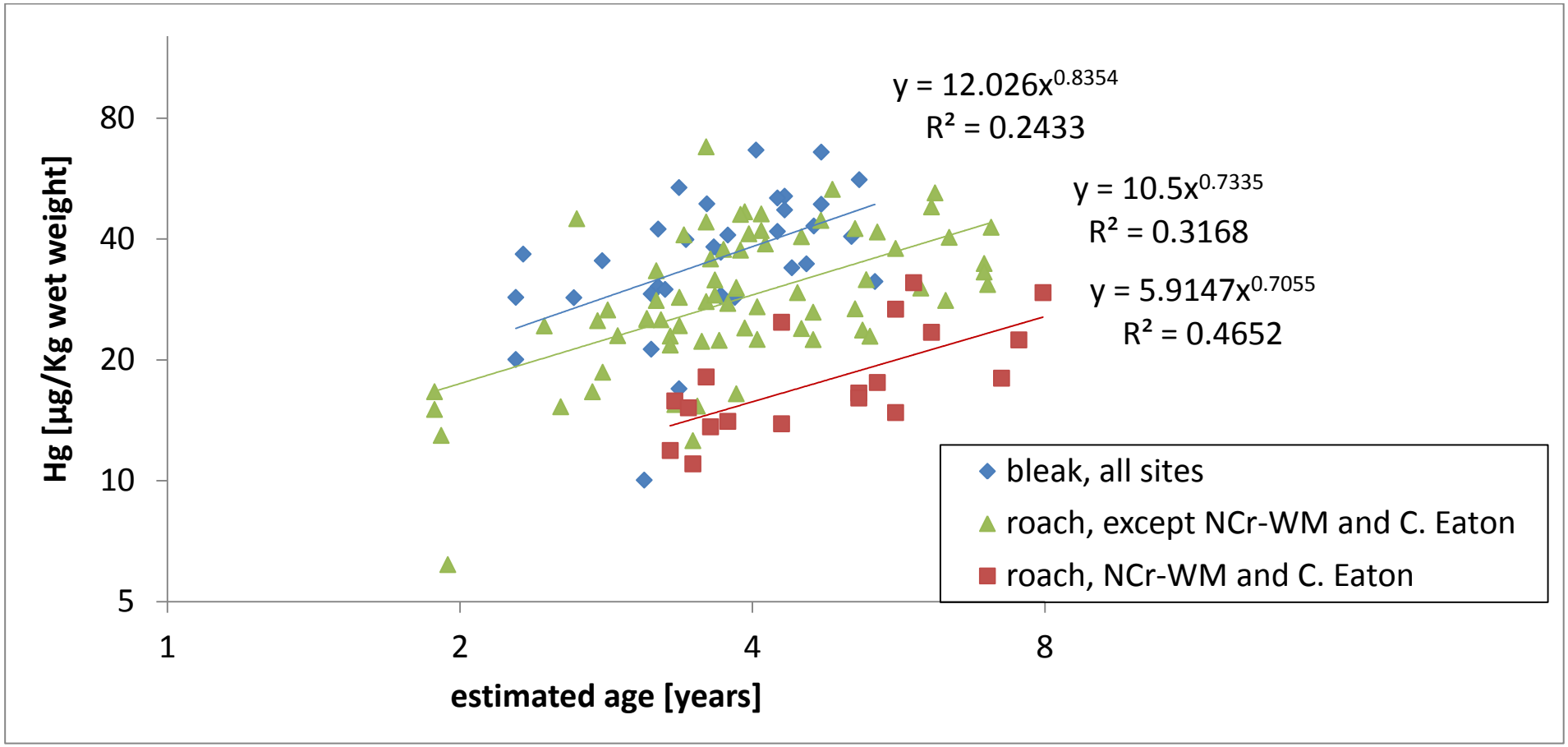


Figure 5 Mercury contamination versus estimated age (from UK reference data (Britton, 2007)) for roach (separated into fish from the two most upstream sites in the Thames catchment: NCr-WM, and the rest) and bleak from the lower Thames (log scale). Site acronyms are explained in table 2.

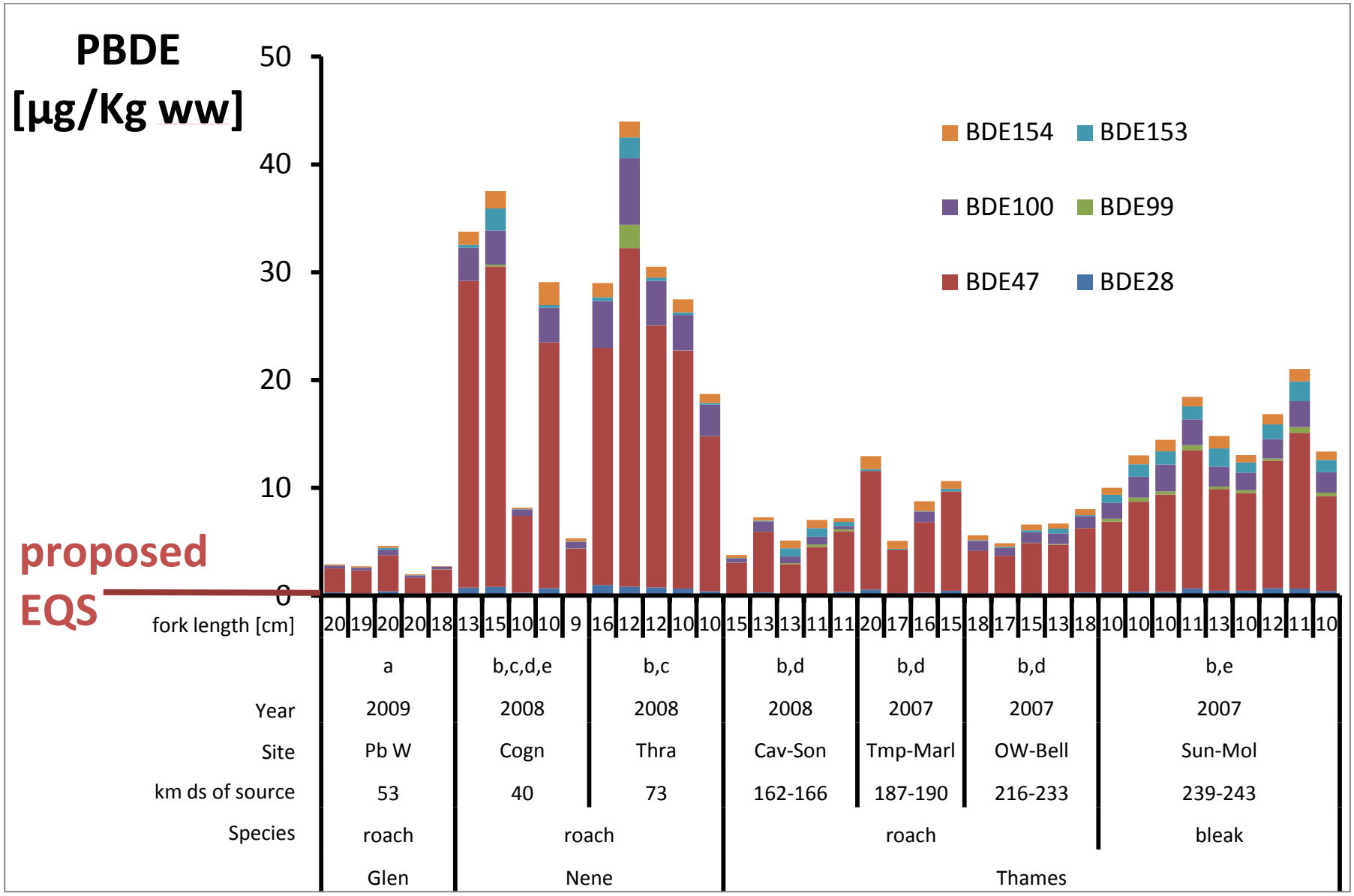
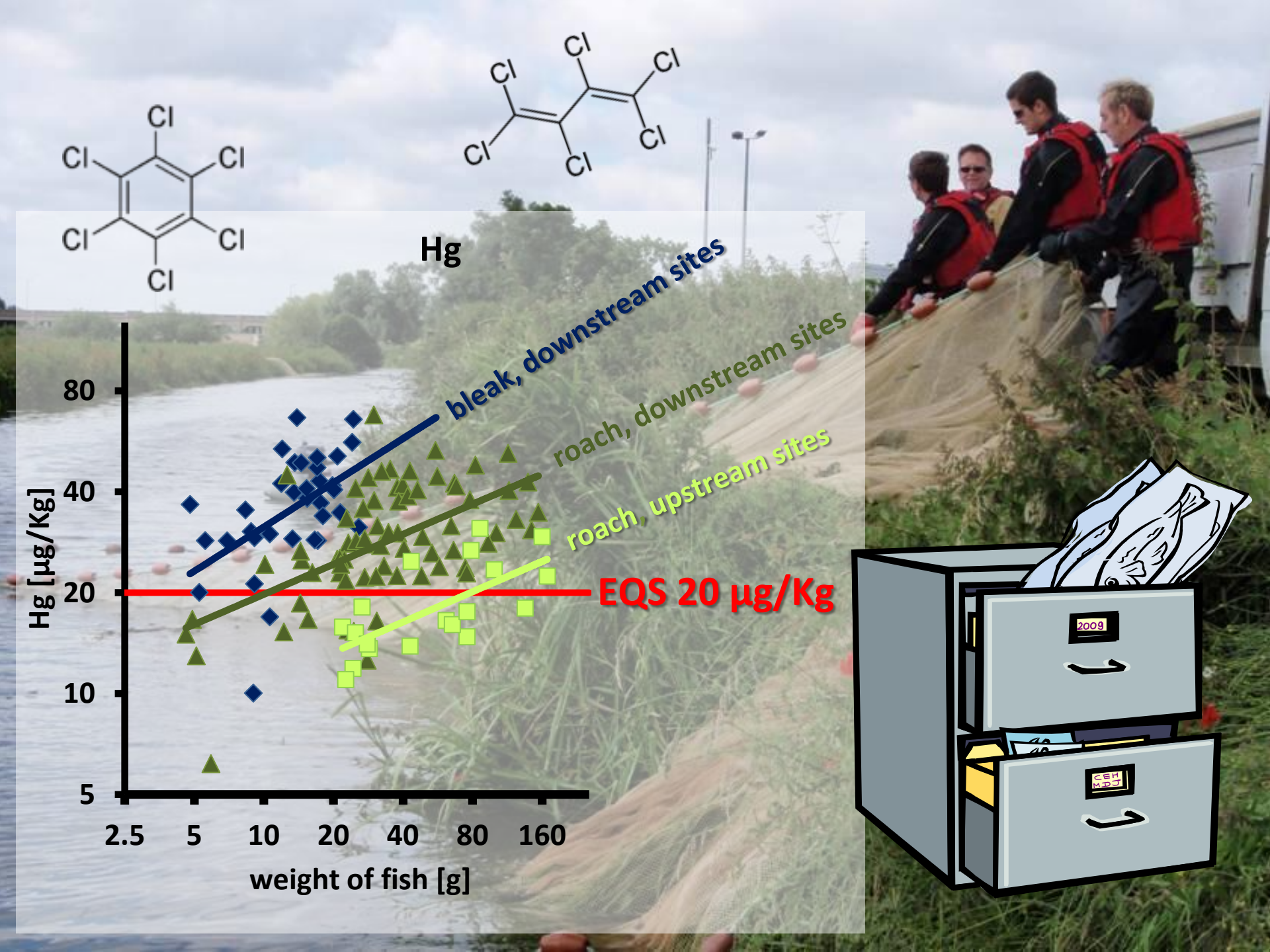


Figure 6 PBDE concentrations in roach and bleak. Different letters denote statistical significant differences (T-test on log transformed data, significance level 5%). Site acronyms are explained in table 2. The proposed EQS is just 0.085 µg/kg as represented by the thick red line.

Highlights

- A fish tissue archive was set up to monitor persistent pollutants in English rivers
- The chemicals with EU EQS for biota (Hg, HCB, and HCBD) were measured in some fish
- Hg concentration was size dependant and exceeded EQS of 20 µg/kg in 79% of samples
- HCB and HCBD were always below their standards of 10 and 55 µg/kg
- A proposed PBDE EQS of 0.0085 µg/Kg was exceeded more than 200 times in every fish


Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Hg
bleak, downstream sites
roach, downstream sites
roach, upstream sites
EQS 20 µg/Kg
Hg [µg/Kg]
80
40
20
10
5
2.5
5
10
20
40
80
160
weight of fish [g]
2009